Evaluated Fate and Effects of Atrazine and Lambda-Cyhalothrin in Vegetated and Unvegetated Microcosms

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ABSTRACT: Contaminants such as nutrients, metals, and pesticides can interact with constructed wetlands and existing drainage ditches used as agricultural best-management practices. Our research has shown that the presence of macrophytes and a hydrologic regime aid in the transfer and transformation of pesticides associated with agricultural runoff. This study consisted of application of both atrazine (triazine herbicide) and lambda-cyhalothrin (pyrethroid insecticide) to vegetated and unvegetated microcosms in order to measure the fate and effects of pesticides applied at suggested field application rates. Exposures focused on monocultures of *Ludwigia peploides* (water primrose) and *Juncus effusus* (soft rush). Pesticide sorption was evident through concentrations of atrazine and lambda-cyhalothrin in plant tissue as high as 2461.4 and 86.50 μ g/kg, respectively. Toxicity was measured in water from unvegetated microcosms for 28 days and in *Chironomus tentans* (midge larvae) exposed to sediment collected from 3 h to 56 days in microcosms receiving the pesticide combination. The comparative survival of test organisms in this study suggests that effective mitigation of pesticides from runoff can depend on the macrophyte contact and vegetative attributes associated with ditches. © 2005 Wiley Periodicals, Inc. Environ Toxicol 20: 487–498, 2005.*

Keywords: aquatic vegetation; atrazine; lambda-cyhalothrin; microcosms; acute toxicity; BMPs;

INTRODUCTION

pesticide fate and effect

The need for ecological testing of pesticide mixtures to predict the effects on aquatic ecosystems receiving agricultural runoff has long been recognized (Cairns, 1983; Fairchild

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et al., 1994). Typically, mixtures may result from either runoff associated with multiple pesticide applications on common acreage during a single growing season or pesticides in confluent runoff that eventually combine in streams. Movement of water-soluble pesticides from the agricultural landscape has resulted in detectable downstream concentrations (USGS, 2000); conversely, transport of hydrophobic chemicals in surface waters following rain events has been attributed either to particle binding with typical field erosive processes or mechanical sheering of soil-bound pesticides under more intense rain events (Ghadiri and Rose, 1991). Schulz et al. (1998) observed that measurable inputs of fewer watersoluble pesticides into surface waters often occur with particle-associated forms. Pesticide concentrations are thought to be greater in eroded sediment than in original soil because of

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TABLE I. Chemical properties of atrazine and lambda-cyhalothrin^{a,b}

Atrazine	Lambda-Cyhalothrin
32	0.005
1.97	3.37
2.34	7.00
30	233
146	62
159	128
4.08×10^{5}	5.629×10^{3}
4.0×10^{-2}	2.0×10^{-4}
	$ \begin{array}{r} 32 \\ 1.97 \\ 2.34 \\ 30 \\ 146 \\ 159 \\ 4.08 \times 10^5 \end{array} $

^aOrme and Kegley, 2003.

uneven distribution of organic matter and sorption within soil aggregates (Ghadiri and Rose, 1991). Regardless of their transport mechanism, the presence of pesticide combinations with differing modes of toxic action, recently described in the literature as across-class mixtures, has resulted in varying toxic responses, which challenge their predictability (Lydy et al., 2004).

Atrazine is the most frequently detected pesticide in the surface waters of the lower Mississippi Valley of the United States (USGS, 2000). This selective herbicide (2-chloro-4-ethylamino-6-isopropylamino-S-triazine) was first registered for use in the United States in 1959 (U.S. EPA, 1994), and its prevalence in surface water reflects its relatively high water solubility, its moderate environmental persistence (Table I), and its usage, which exceeds 28 million kg/year (NASS, 2002). As well, concern for atrazine's effect on nontarget organisms has required that subsequent risk assessments in aquatic systems account for residence times in static surface- and groundwater (Solomon et al., 1996; Dodson et al., 1999).

Lambda-cyhalothrin [(RS)- α -cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoropropenyl)-2,2,-dimenthylcyclo-propanecarboxylate] is a frequently used synthetic pyrethroid with an applied rate of 25,000 kg/year in 2001 (NASS, 2002). The associated physical and chemical properties of synthetic pyrethroids (Table I) result in a perceived low potential for environmental exposure because of their relatively short residence time in surface waters (Hand et al., 2001). Exceptional hydrophobicity results in rapid partitioning from the water column, low mammalian and avian toxicity, and relatively short environmental half-lives. However, pyrethroid toxicity measured with aquatic invertebrates in laboratory conditions (Maund et al., 2002; Orme and Kegley, 2003) has led to a number of complex ecological risk assessments.

Constructed wetlands and agricultural drainage ditches in the lower Mississippi Valley serve to receive and convey diverse types of discharge from agricultural production areas to receiving bodies. Attributes of these systems capable of modifying runoff include vegetation, hydraulic residence time, sediment interactions, and the cumulative effect of distance from point of input (Moore et al., 2001; Bouldin et al., 2004b; Cooper et al., 2004; Milam et al., 2004). The physical, chemical, and biological characteristics of these systems combine to enhance mitigation of the effects of agriculturalassociated contaminants. To distinguish some of the complex interactions within the water, sediment, and plant matrices, microcosms possessing attributes common to these systems can be utilized in the assessment of runoff (Catallo et al., 1999). Many published toxicological end points have been derived from the study of laboratory exposures of single compounds, whereas investigation of pesticide mixtures sometimes has resulted in end points that differ from those from single-dose exposures (Anderson and Lydy, 2002; Jin-Clark et al., 2002). Able to simulate realistic scenarios of biological, physical, and chemical interactions with compound mixtures, microcosms can facilitate an examination of how various factors may combine to affect the fate and effects of pesticides common in the lower Mississippi Valley.

This microcosm study used vegetated and unvegetated treatments to determine the fate and effects of atrazine and lambda-cyhalothrin separately and in combination. These exposures were used to characterize the pesticides, as well as the biological and physical interactions, in agricultural receiving systems.

MATERIALS AND METHODS

Microcosm studies during the summer of 2003 utilized 16 Rubbermaid[®] polypropylene containers (42 L in volume and $40.6 \times 50.8 \times 22.9$ cm in size). Ditch sediment with overlying dechlorinated municipal tap water was introduced into 12 containers, of which four were monocultures of J. effusus and four were monocultures of L. peploides, with the remaining microcosms filled with sediment from an adjacent agricultural field. No detectable atrazine or lambda-cyhalothrin was measured in either type of sediment prior to application of either pesticide. Prior to study, similar vegetative and chemical conditions in the microcosms were established in a greenhouse environment for 2 months with 16 h:8 h light-dark photoperiod and average light intensity of 22,000-45,000 lux. During that time unvegetated microcosms were periodically cleared of any macrophytes and algae, and any extraneous vegetation was cleared from monoculture microcosms. Vegetative cover was approximately 95%, and sediment and water depths were approximately 10.0 and 8.0 cm, respectively.

Greenhouse temperature was regulated with an evaporative cooling system that maintained air temperatures of approximately 29°C–30°C and relative humidity of 60%–70%. The overlying dechlorinated water was Jonesboro, Arkansas, municipal tap water filtered through a carbon filter bed.

^bUSDA ARS, 2003.

Treatment Applications

The pesticide treatments were prepared and mixed with dechlorinated municipal tap water in 1-L glass beakers at concentrations targeted for recommended field application of atrazine as Aatrex[®] [2.23 kg active ingredient (a.i.)/ha] and lambda-cyhalothrin as Karate[®] (0.028 kg a.i./ha). The treatments were immediately introduced to the overlying water and gently agitated with treatment beakers to simulate a 0.64-cm precipitation event from a 2.03-ha contributing area.

The four microcosm types were the control (untreated, receiving no pesticide application), atrazine at 2.23 kg a.i./ha, lambda-cyhalothrin at 0.028 kg a.i./ha, and atrazine combined with lambda-cyhalothrin at the same application rates as those in each vegetated monoculture, as well as in each of two sediments with no vegetation.

Sediment Particle Size

Particle-size composition of each type of sediment was analyzed prior to pesticide application according to the method of Gee and Bauder (1986).

Physicochemical Parameters

Water quality parameters such as temperature, pH, dissolved oxygen, carbonate alkalinity, hardness, and conductivity were measured prior to and concurrent with pesticide and toxicity sampling. All water quality analyses followed American Public Health Association (APHA, 1998) guidelines, utilizing a YSI Model 610 multimeter and an Accumet AR 25 dual-channel pH and ammonia meter.

Pesticide Analyses

Chemical analyses were conducted on unfiltered water, sediment, and plants to determine pesticide concentrations throughout the exposure periods as described by Bennett et al. (2000), Cooper et al. (2004), and Smith et al. (2004). Atrazine and lambda-cyhalothrin were quantified with a HP 6890 gas chromatograph equipped with a 30-m HP-5MS column. The oven temperature program was as follows: 85°C held for 1 min; to 190°C at a rate of 25°C/min, held for 25 min; and then to 230°C at 25°C/min, held for 30 min. Injector and detector temperatures were set to 250°C and 320°C, respectively. Ultra-high-purity helium, the carrier gas (nexAir, Memphis, TN, USA), was set to a constant flow of 28 cm/s and ultra-high-purity nitrogen, the makeup gas (Whatman Nitrogen Generator) was set at a constant makeup flow of 40 mL/min. A multilevel calibration procedure was utilized with standards and updated every ninth sample. The limits of detection (LOD) for atrazine in water, sediment, and plants were 0.01 μ g/L, 0.1 μ g/kg, and $0.1 \mu g/kg$, respectively; in addition, the limit of quantitation (LOQ) for atrazine in water was 0.1 μ g/L. The LOD for lambda-cyhalothrin in water, sediment, and plants were $0.001 \mu g/L$, $0.01 \mu g/kg$, and $0.01 \mu g/kg$, respectively; in addition, the LOQ for lambda-cyhalothrin in water was $0.01 \mu g/L$. Mean extraction efficiencies based on fortified samples were >90% for water, sediment, and plants.

Aqueous grab samples and sediment were collected prior to exposure in order to have background measurements of chemistry and acute toxicity for all exposures. Sample collection included water collected 3 and 24 h and 7, 14, and 28 days postapplication. Also, for any water sample that elicited a response statistically different from the controls at 14 or 28 days postapplication, additional samples were taken 56 days after dosing. Sediment and plant tissue were collected at 3 and 24 h and 7, 14, and 28 days postapplication and also, only from microcosms with aqueous concentrations eliciting a statistically different response from controls at 14 or 28 days, at 56 days postapplication. Water, sediment, and plants were composited from at least three areas of the microcosm, and water samples were extracted on site with ethyl acetate and potassium chloride (Bennett et al., 2000). For toxicity and pesticide analyses, sediment was collected from the upper 3 cm, whereas plant tissue was extracted from submerged stems and leaves.

Toxicity Testing

Forty-eight-hour acute toxicity in the aqueous samples was assessed with Ceriodaphnia dubia (water flea) and Pimephales promelas (fathead minnow) following methods outlined by the U.S. EPA (2002). Inhibition of Chironomus tentans survival and growth was assessed in solid-phase 10day sediment tests (U.S. EPA, 2000). Overlying water was renewed in test chambers twice daily using a static flowthrough system. In addition, organisms in each test chamber were fed 1 mL of Tetramin® solution (4 g/L) daily during the 10 days. To determine overlying water quality, parameters including temperature (°C), dissolved oxygen (mg/L), conductivity (μ S/cm), and pH were measured at regular intervals in randomly selected test chambers of each site (APHA, 1998). Results from the toxicity assays were statistically analyzed using Toxcalc® (version 5.0.25). All data were tested using $\alpha = 0.05$, and the normality assumption was tested using Shapiro-Wilk's test and significance of survival was determined using Steel's Many-One Rank test.

RESULTS

Sediment Particle Size

Sediment samples were composed primarily of silt (field— 93.0%; ditch—94.1%), and particle size distribution differed in that field sediment included a slightly larger

TABLE II. Measured physicochemical parameters (± 1 SD) of overlying water in microcosms amended with atrazine and lambda-cyhalothrin

Amendment	Micro	ocosm	pH n = 7	Oxygen (mg/L) $n = 7$	Conductivity $(\mu s/cm)$ $n = 7$	Alkalinity (mg/L) $n = 1$	Hardness (mg/L) $n = 1$	Water Temperature (°C) $n = 7$
Atrazine	Vegetated	L. peploides	7.31 ± 0.52	6.5 ± 0.9	249 ± 133	74	70	27.0 ± 2.2
		J. effusus	7.25 ± 0.41	7.0 ± 1.0	206 ± 63	40	110	26.6 ± 1.5
	Unvegetated	Ditch sediment	7.46 ± 0.31	7.4 ± 0.9	184 ± 33	64	150	27.1 ± 1.8
		Field sediment	7.76 ± 0.21	6.7 ± 1.0	263 ± 103	102	80	27.2 ± 2.1
Lambda-	Vegetated	L. peploides	7.04 ± 0.57	6.9 ± 0.9	93 ± 37	36	20	26.7 ± 1.6
cyhalothrin		J. effusus	7.38 ± 0.35	7.8 ± 0.8	227 ± 93	62	60	27.4 ± 2.2
	Unvegetated	Ditch sediment	8.12 ± 0.86	7.5 ± 1.2	173 ± 19	54	70	27.2 ± 2.3
		Field sediment	8.06 ± 0.47	7.3 ± 1.3	266 ± 48	110	110	26.9 ± 1.9
Combination	Vegetated	L. peploides	7.39 ± 0.45	7.5 ± 0.5	159 ± 95	32	60	27.3 ± 2.3
		J. effusus	7.61 ± 0.39	7.5 ± 0.7	280 ± 55	100	90	27.5 ± 1.5
	Unvegetated	Ditch sediment	7.60 ± 0.27	7.3 ± 0.6	216 ± 25	98	100	27.1 ± 1.9
		Field sediment	7.99 ± 0.13	7.5 ± 0.8	228 ± 95	110	110	27.6 ± 2.2
Unamended	Vegetated	L. peploides	6.67 ± 0.80	6.8 ± 1.1	77 ± 33	28	20	27.0 ± 2.2
	_	J. effusus	7.44 ± 0.68	7.1 ± 1.0	192 ± 61	68	90	27.4 ± 1.5
	Unvegetated	Ditch sediment	8.43 ± 0.96	8.4 ± 1.0	182 ± 38	72	80	27.2 ± 2.0
		Field sediment	8.50 ± 0.66	7.8 ± 1.2	216 ± 57	122	130	27.1 ± 1.5

fraction of clay than did ditch sediment (5.8% and 2.7%, respectively).

Physicochemical Parameters

Average pH and hardness in the overlying water from the 2003 microcosms tended to be lowest in those microcosms containing L. peploides (pH = 6.67–7.39; hardness = 20–70 mg/L; Table II), whereas the pH in the overlying water from the remaining microcosms tended to be higher in unvegetated treatments (7.46–8.50). The mean DO ranged from 6.5 to 8.4 mg/L.

Pesticide Analyses

The aqueous concentration of atrazine was highest after 28 days and of lambda-cyhalothrin after 3 h. An atrazine concentration of 112.94 μ g/L was measured in a pesticide combination—amended microcosm (Table III), whereas a lambda-cyhalothrin concentration of 28.282 μ g/L was measured in the *J. effusus* microcosm exposed only to that pesticide. Although atrazine concentrations were detectable only in water sampled after 28 and 56 days in microcosms amended only with that compound, concentrations were detected

within 3–24 h in water from microcosms amended with the pesticide combination. Conversely, in all microcosms dosed with lambda-cyhalothrin, in which that pesticide was partitioned from the water column within 24 h–7 days, concentrations of 0.038 µg/L or less were detected after 7 days.

The highest pesticide concentrations in sediment were collected from pesticide-combination-amended microcosms. An atrazine concentration of 2082.7 μ g/kg was measured in sediment from an unvegetated microcosm collected at 24 h, whereas higher lambda-cyhalothrin concentrations were measured at 7 days from a vegetated microcosm receiving the same amendment (136.78 μ g/kg; Table III). Plant tissue analyzed from microcosms had peak atrazine concentrations in *L. peploides* of 2461.4 μ g/kg 7 days after amendment with that compound (Table III), and the highest lambda-cyhalothrin concentration in tissue from *L. peploides* was detected at 24 h from the microcosm exposed to the pesticide combination (86.50 μ g/kg).

Toxicity Testing

Survival of *P. promelas* was reduced at 3 and 24 h in water from the atrazine-amended microcosms (Table IV). In addition, *C. dubia* survival was reduced in water sampled at 3 h

TABLE III. Measured pesticide concentrations of mesocosms following pesticide application (aqueous concentrations reported in μ g/L; sediment and plant concentrations reported in μ g/kg)

						Tiı	me Postappl	ication		
Amendment	Microcosm	Pesticide		0 h	3 h	24 h	7 Days	14 Days	28 Days	56 Days
Atrazine	J. effusus	Atrazine	Water	nd	nd	nd	nd	nd	5.8	ns
			Sediment	nd	296.1	1902.6	228.2	80.8	nd	nd
			Plant	ns	nd	41.1	6.4	4.0	nd	ns
		Lambda-cyhalothrin	Water	nd	0.601	0.031	nd	nd	nd	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
			Plant	ns	nd	nd	nd	nd	nd	ns
	L. peploides	Atrazine	Water	nd	nd	nd	nd	nd	nd	101.62
			Sediment	nd	320.5	752.1	392.6	556.7	138.5	81.9
			Plant	ns	75.9	nd	2461.4	449.4	687.0	ns
		Lambda-cyhalothrin	Water	nd	4.003	nd	nd	nd	nd	nd
			Sediment	nd	nd	nd	nd	nd	nd	nd
			Plant	ns	nd	nd	nd	nd	nd	ns
	Field sediment	Atrazine	Water	1.83	nd	nd	nd	nd	nd	ns
			Sediment	nd	96.5	196.8	531.8	251.5	130.3	nd
		Lambda-cyhalothrin	Water	nd	nd	0.001	nd	nd	nd	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
	Ditch sediment	Atrazine	Water	0.50	nd	nd	nd	nd	22.97	24.09
			Sediment	nd	56.2	1731.8	861.1	1213.5	3.1	26.9
		Lambda-cyhalothrin	Water	nd	0.106	0.002	nd	nd	0.002	0.008
			Sediment	nd	nd	nd	nd	nd	nd	nd
Lambda-	J. effusus	Atrazine	Water	nd	2.18	11.40	2.70	0.37	0.91	ns
cyhalothrin			Sediment	nd	49.8	nd	nd	nd	nd	nd
			Plant	ns	nd	nd	nd	nd	nd	ns
		Lambda-cyhalothrin	Water	nd	28.282	0.122	0.006	nd	0.021	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
			Plant	ns	0.20	nd	1.33	nd	nd	ns
	L. peploides	Atrazine	Water	nd	1.88	0.46	1.79	nd	8.77	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
			Plant	ns	nd	nd	11.7	nd	nd	ns
		Lambda-cyhalothrin	Water	nd	4.209	0.088	nd	nd	0.009	ns
			Sediment	nd	nd	nd	nd	0.26	nd	nd
			Plant	ns	nd	46.90	46.77	0.83	nd	ns
	Field sediment	Atrazine	Water	0.59	31.04	3.06	9.82	4.43	4.57	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
		Lambda-cyhalothrin	Water	nd	6.122	1.523	0.018	nd	0.037	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
	Ditch sediment	Atrazine	Water	0.02	2.58	2.79	3.73	4.74	2.07	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
		Lambda-cyhalothrin	Water	nd	5.651	0.384	nd	0.003	nd	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
Combination	J. effusus	Atrazine	Water	nd	2.22	nd	nd	nd	nd	ns
			Sediment	nd	158.4	725.7	684.1	776.5	164.7	38.6
			Plant	ns	0.0	197.7	31.0	343.4	nd	ns
		Lambda-cyhalothrin	Water	nd	nd	0.245	0.001	nd	nd	ns
			Sediment	nd	nd	1.54	nd	0.11	40.13	nd
			Plant	ns	6.84	4.69	19.82	0.21	nd	ns
	L. peploides	Atrazine	Water	nd	nd	nd	74.85	nd	112.94	ns
			Sediment	nd	333.6	1281.6	716.3	304.9	103.6	36.6
			Plant	ns	153.0	426.2	64.7	63.7	26.0	ns
		Lambda-cyhalothrin	Water	nd	2.33	0.03	0.09	nd	nd	ns
			Sediment	nd	3.31	4.55	136.78	nd	nd	0.09
			Plant	ns	27.45	86.50	8.92	nd	nd	ns

(Continued.)

TABLE III. (Continued)

						Time Pos	tapplicatio	n		
Amendment	Microcosm	Pesticide		0	3 h	24 h	7 Days	14 Days	28 Days	56 Days
	Field sediment	Atrazine	Water	0.45	nd	8.20	nd	nd	nd	ns
			Sediment	nd	nd	245.8	391.5	568.8	426.4	75.3
		Lambda-cyhalothrin	Water	nd	14.813	3.956	0.035	nd	nd	ns
			Sediment	nd	0.00	69.78	4.10	nd	nd	nd
	Ditch sediment	Atrazine	Water	nd	nd	nd	nd	nd	nd	nd
			Sediment	nd	404.0	2082.7	746.0	1231.1	309.0	373.5
		Lambda-cyhalothrin	Water	nd	11.048	1.336	nd	0.003	nd	nd
			Sediment	0.00	67.22	9.08	3.15	0.77	nd	nd
Unamended	J. effusus	Atrazine	Water	nd	nd	nd	3.53	0.32	0.80	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
			Plant	ns	nd	nd	nd	nd	nd	ns
		Lambda-cyhalothrin	Water	nd	nd	nd	nd	0.038	nd	ns
			Sediment	nd	nd	nd	nd	nd	nd	4.30
			Plant	ns	nd	nd	nd	nd	nd	ns
	L. peploides	Atrazine	Water	nd	a	nd	nd	0.60	3.05	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
			Plant	ns	nd	nd	nd	nd	nd	ns
		Lambda-cyhalothrin	Water	nd	a	nd	nd	nd	0.021	ns
			Sediment	nd	nd	3.02	nd	90.13	nd	nd
			Plant	ns	nd	nd	nd	nd	nd	ns
	Field sediment	Atrazine	Water	2.27	1.62	1.70	0.99	4.53	1.66	ns
			Sediment	nd	nd	nd	nd	nd	nd	nd
		Lambda-cyhalothrin	Water	nd	nd	nd	nd	nd	nd	ns
		•	Sediment	nd	nd	nd	nd	nd	nd	nd
	Ditch sediment	Atrazine	Water	0.49	1.97	1.50	nd	2.72	1.66	ns
			Sediment	nd	nd	nd	nd	nd	nd	35.5
		Lambda-cyhalothrin	Water	nd	nd	nd	nd	nd	0.001	ns
		•	Sediment	nd	nd	nd	nd	nd	nd	nd

^aBroken in transport.

ns = not sampled for GC analysis. nd = not detected (below LOD). LOD of atrazine in water, sediment, and plants = $0.01~\mu g/L$, $0.1~\mu g/kg$, and $0.1~\mu g/kg$, respectively. LOD of lambda-cyhalothrin in water, sediment, and plants = $0.001~\mu g/L$, $0.01~\mu g/kg$, and $0.01~\mu g/kg$, respectively.

except from the vegetated microcosm containing J. effusus. Although all C. dubia survived exposure to water from this microcosm at 3 h, survival in water collected at 24 h was reduced to 45%. Survival was not reduced in water collected at 7 days; however, P. promelas experienced mortality in both vegetated and unvegetated microcosms at the 14-day sampling. A similar impact on C. dubia survival (survival reduced to 60% of that of the control) was measured in water from the unvegetated microcosm. No P. promelas survived exposure to water from microcosms amended with lambda-cyhalothrin through 3 h, and significant toxicity to C. dubia (<10% survival) was measured through 24 h. None of the P. promelas or C. dubia survived in water collected at 3 h from pesticidecombination-amended microcosms, and although none of the C. dubia survived in water collected at 24 h postapplication, survival of 72.5% of the P. promelas from the vegetated microcosms of the same sample was measured.

Sediment collected from atrazine-amended microcosms reduced survival of *C. tentans* at 24 h only from the microcosm containing *J. effusus* (Table V). Responses of sedi-

ment from this microcosm also were measured at 14 and 28 days. Either lethal or sublethal effects were measured through 14 days postapplication in *C. tentans* exposed to sediment from lambda-cyhalothrin-dosed microcosms. In addition, all sediment samples from lambda-cyhalothrin-amended microcosms, except for that from an unvegetated microcosm at 7 days, continued to show impairment. Sediment from all pesticide-combination-amended microcosms significantly reduced either the survival or growth of *C. tentans* through 7 days. Significant lethal or sublethal effects continued to be measured through 56 days in sediment sampled from the combination-amended microcosm containing *J. effusus*.

DISCUSSION

Temporal patterns of toxicity reduction have provided evidence of vegetative mitigation of pesticides (Schulz et al., 2003; Bouldin et al., 2004b; Milam et al., 2004). In this

TABLE IV. Survival (±1 SD) of test organisms exposed to water from microcosms following pesticide application

					T	Time Postapplication	uo		
Amendment	Microcosm		0	3 h	24 h	7 Days	14 Days	28 Days	56 Days
Atrazine	J. effusus	P. promelas	100 ± 0.0	35 ± 7.7^{a}	60 ± 5.7^{a}	+1	97.5 ± 5.8	100 ± 0.0	ns
		C. dubia	+1	100 ± 0.0	$45 \pm 12.4^{\rm a}$		+1	100 ± 0.0	ns
	L. peploides	P. promelas		42.5 ± 5.9^{a}	42.5 ± 9.2^{a}	+1	+1	95 ± 6.7	85 ± 13.0
	,	C. dubia	+1	65 ± 14.0^{a}	85 ± 8.7	95 ± 8.8	+1	100 ± 0.0	100 ± 0.0
	Ditch sediment	P. promelas		40 ± 8.5^{a}	47.5 ± 6.0^{a}	+1		95 ± 10.8	ns
		C. dubia	+1	+1	95 ± 8.8	+1	+1	+1	ns
	Field sediment	P. promelas	+1	+1	47.5 ± 12.6^{a}	+1	+1	+1	95 ± 6.7
		C. dubia	100 ± 0.0	60 ± 11.6^{a}	85 ± 16.0	100 ± 0.0	60 ± 20.8^{a}	100 ± 0.0	100 ± 0.0
Lambda-cyhalothrin	J. effusus	P. promelas	+1	0 ± 0.0^{a}	92.5 ± 14.9	+1	+1	+1	ns
		C. dubia	+1	0 ± 0.0^{a}	10 ± 6.7^{a}	+1	+1	± 1	ns
	L. peploides	P. promelas	+1	0 ± 0.0^{a}	92.5 ± 14.9	+1	+1	+1	su
		C. dubia	+1	+1	5 ± 2.1^{a}	+1	+1	+1	su
	Ditch sediment	P. promelas	+1	+1	100 ± 0.0	+1	+1	+1	su
		C. dubia	+1	+1	0 ± 0.0^{a}	+1	+1	+1	ns
	Field sediment	P. promelas	+1	+1	95 ± 12.0	+1	+1	+1	ns
		C. dubia	+1	+1	0 ± 0.0^{a}	+1	+1	+1	ns
Combination	J. effusus	P. promelas	100 ± 0.0	0 ± 0.0^{a}	15 ± 10.5^{a}	+1	87.5 ± 17.5	97.5 ± 5.8	ns
		C. dubia	+1	+1	0 ± 0.0^{a}	+1	+1	+1	ns
	L. peploides	P. promelas	+1	+1	72.5 ± 10.9^{a}	+1	+1	+1	ns
		C. dubia	+1		0 ± 0.0^{a}	+1	+1	+1	us
	Ditch sediment	P. promelas	100 ± 0.0		0 ± 0.0^{a}	+1	+1	+1	us
		C. dubia	100 ± 0.0	+1	0 ± 0.0^{a}	+1	+1	+1	ns
	Field sediment	P. promelas	+1	+1	0 ± 0.0^{a}	+1	+1	+1	55 ± 12.9^{a}
		C. dubia	+1	+1	0 ± 0.0^{a}	+1	+1	95 ± 8.8	100 ± 0.0
Unamended	J. effusus	P. promelas	+1	+1	+1	+1	+1	+1	ns
		C. dubia	+1	+1	85 ± 16.0	+1	+1	+1	su
	L. peploides	P. promelas	+1	97.5 ± 5.8	+1	+1	87.5 ± 10.4	+1	su
		C. dubia	+1	+1	± 1	+1	+1	+1	su
	Ditch sediment	P. promelas	+1	95 ± 10.8	+1	+1	+1	100 ± 0.0	ns
		C. dubia	+1	+1	+1	+1	+1	+1	su
	Field sediment	P. promelas	+1	100 ± 0.0	100 ± 0.0	95 ± 10.8	90 ± 14.1	+1	su
		C. dubia	+1	+1	+1	+1	+1	+1	su
	Laboratory control	P. promelas	+1	100 ± 0.0	100 ± 0.0	100 ± 0.0	+1	100 ± 0.0	100 ± 0.0
		C. dubia	+1	100 ± 0.0	100 ± 0.0	90 ± 10.1	+1	100 ± 0.0	100 ± 0.0
	,								

 $^{\rm a}$ Significantly different from control at $\alpha=0.05.$ ns = not sampled.

TABLE V. Survival and growth as milligrams (±1 SD) of C. tentans exposed to sediment from microcosms following pesticide application

Amendment Microcosm Atrazine L. peploides J. effusus Ditch sediment Field sediment Field sediment J. effusus Ditch sediment Field sediment Field sediment Field sediment Field sediment Field sediment Ditch sediment J. effusus J. effusus J. effusus J. effusus Ditch sediment Field sediment Ditch sediment Ditch sediment J. effusus		0^{a} 100 ± 0.0	3 h	24 h	7 Days	14 Days	28 Days	56 Days
		100 ± 0.0	4					
			-		95 ± 10.8		50 ± 17.5^{b}	100 ± 0.0
		2.01 ± 0.25	+1	+1	+1		+1	1.18 ± 0.12
		+1			+1		+1	+1
		+1	+1	+1	+1	+1	+1	+1
		100 ± 0.0		85 ± 18.1	85 ± 18.1	+1	70 ± 18.9^{b}	100 ± 0.0
		+1	+1	+1	+1	+1	+1	1.42 ± 0.51
	Growth Survival Growth	+1	100 ± 0.0	90 ± 12.6	+1	+1	+1	+1
	Survival Growth	+1		+1	+1	+1	1.87 ± 0.39	+1
	Growth	+1	$40 \pm 25.3^{\rm b}$	± 1	$62.5 \pm 6.8^{\rm b}$	+1	+1	+1
		+1	2.10 ± 0.60	$1.27 \pm 0.46^{\rm b}$	+1	+1	+1	+1
	Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	+1	+1	+1	+1	+1	+1	+1
	t Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	+1	+1	+1	+1	+1	+1	+1
	t Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	2.31 ± 0.44	1.50 ± 0.62	1.43 ± 0.68	1.86 ± 0.58	$1.05 \pm 0.62^{\rm b}$	1.23 ± 0.21^{b}	1.53 ± 0.29
	Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	+1	+1	+1	+1	+1	+1	+1
	Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	+1	+1	+1	+1	+1	+1	+1
	t Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	+1	+1		+1	+1	+1	+1
	t Survival	+1	+1	+1	+1	+1	+1	+1
	Growth	+1	2.02 ± 0.28	+1	+1	+1	+1	+1
J. effusus Ditch sediment	Survival	+1	+1	+1	+1	± 1	+1	+1
J. effusus Ditch sediment	Growth	2.01 ± 0.25	+1	2.12 ± 0.23	+1	+1	+1	+1
Ditch sediment	Survival	+1	+1	+1	+1	+1	+1	+1
Ditch sediment	Growth	+1		1.97 ± 0.31	+1	+1	+1	+1
	t Survival	+1	+1	+1		100 ± 0.0	+1	+1
	Growth	+1	2.26 ± 0.75	+1	+1	+1	2.45 ± 0.30	1.10 ± 0.17
Field sediment	t Survival	+1	100 ± 0.0	100 ± 0.0	+1	85 ± 18.1	+1	+1
	Growth	+1	+1	+1		+1	+1	2.00 ± 0.29
Laboratory control	ntrol Survival	100 ± 0.0	100 ± 0.0	+1		+1	100 ± 0.0	+1
	Growth	+1	+1	1.97 ± 0.29	2.27 ± 38.7	1.86 ± 0.19	+1	1.40 ± 0.37

 a Background toxicity obtained for composite field samples. b Significantly different from control at $\alpha=0.05$.

study, it was found that senescence of dicotyledonous vegetation (L. peploides) occurred in microcosms exposed to atrazine (both alone or in combination with lambda-cyhalothrin) following tissue accumulation. However, lower atrazine accumulation was measured in tissue from J. effusus, which was less affected by the herbicide. This accumulation and resilience to pesticide mixtures was noted by Lytle and Lytle (2002), who further suggested the use of J. effusus in phytoremediation systems.

Repeated exposure of resident macrophyte communities to atrazine could result in dieback of atrazine-sensitive vegetation and tolerant monocotyledonous communities that could include J. effusus. Structural and functional effects on aquatic ecosystems have been observed in aqueous atrazine concentrations of as little as 10 μ g/L (U.S. EPA, 2003). Although pond mesocosms dosed with atrazine may result in a significantly altered species composition of macrophyte communities, the associated total biomass and primary production can be less affected through species substitution (Fairchild et al., 1994). Maintenance of ecosystem function through replacement of sensitive species with more tolerant varieties was described by Cairns and Dickson (1977). Subsequent "functional redundancy" has been noted in stressed macrophyte and algal communities exposed to atrazine (DeNoyelles et al., 1982; Schmitt-Jansen, 2005). Although senescence of atrazine-sensitive vegetation was observed in this study, shortened exposure time, vegetative monocultures, and enclosed systems prevented the establishment of more tolerant macrophytes, as observed by Fairchild et al. (1994).

Water/macrophyte contact has been shown to be of significance in the effective mitigation of the effects of pesticides in drainage ditches (Moore et al., 2001). Other studies found that in vegetated systems less time was required to mitigate the effects of pesticides by their reduction to a level to that did not acutely affect aquatic organisms (Schulz et al., 2003; Milam et al., 2004). Also noted has been the importance of retention time for various pesticide uptake rates by macrophytes (Lytle and Lytle, 2002). In this study the accumulation of pesticides in plant tissues demonstrated the significance of water/macrophyte contact in systems receiving runoff. Although eventually a reduction in toxicity in unvegetated microcosms was measured, more rapid mitigation of toxic effects was measured in microcosms containing macrophytes. These findings support those of Runes et al. (2001), who demonstrated that sediments act to partition atrazine. It seems that attributes associated with macrophytes such as sorption to organic matter and increased microbial activity enhanced its ability to mitigate the effects of the pesticide (Huckins et al., 1986; Chung et al., 1996). Additionally, increased organic matter is known to enhance the sorption of hydrophobic compounds (Zhou et al., 1995; Hand et al., 2001), resulting in partitioning of lambda-cyhalothrin to plant roots and associated rhizospheres.

Although controlled laboratory studies were used to establish the importance of aquatic vegetation in pesticide mitigation (Karen et al., 1998; Hand et al., 2001), more recent field studies have assessed mitigation of atrazine and pyrethroid concentrations in constructed wetlands and vegetated drainage ditches (Moore et al., 2001; Moore et al., 2002; Bouldin et al., 2004b; Cooper et al., 2004). Field studies with realistic exposure regimes would be expected to better predict adverse impacts of pesticides, even though most outdoor simulations have only elaborated on pond mesocosms or littoral enclosures (Graney et al., 1995). Exposures such as these may have lacked attributes common to agricultural receiving systems (e.g., consistent water and sediment depth, flow, and spatial distance from point of input).

Lentic systems used for pesticide exposures lacking uniform water depth in the littoral zone may create plant zonation and result in variable macrophyte/pesticide contact. Consistent water depth and plant cover in greenhouse microcosms such as those described in this study provide water/macrophyte and water/sediment associations that simulate receiving systems. However, pond exposures and static microcosms fail to account for the influence of flow on agricultural receiving systems. Presence of channelized stream flow in mitigation wetlands and agricultural conveyance structures increases water/macrophyte contact. A stream flow of sufficient rate is calculated with measured velocity; otherwise, a stream flow with known channel slope and hydraulic roughness is calculated with the Manning equation (Mitsch and Gosselink, 2000). Streambed roughness and the proportion of flow in contact with the streambed reduce water velocity in agricultural drainage ditches and constructed wetlands. Attributes of vegetated structures include litter and stems from macrophytes that provide dominant drag forces and increase the Manning coefficient (n) by a factor of 10-20 (Kadlec and Knight, 1996). Decreased flow increases retention time and water/ macrophyte contact in agricultural drainage systems and removes suspended solids from the water column (Bouldin et al., 2004a). Removal of water-soluble as well as particulate-bound hydrophobic compounds is accomplished by vegetative communities through water/macrophyte contact and precipitation of suspended sediment.

Bouldin et al. (2004a) described resident macrophyte communities and suggested the optimization of vegetative attributes in agricultural drainages as an attainable best management practice (BMP); additionally, they reported that forested or grassed buffer strips (5–8 m) concurrent with vegetated drainage ditches provided mitigation areas that reduced agricultural non-point source pollution. Van Strien et al. (1991) noted that ditch maintenance every 2–3 years sustained high macrophyte richness and recommended placement of this nutrient-rich organic sediment on adjacent fields. Sediment- or macrophyte-bound pesticides remaining in this organic matter would continue to degrade through microbial and photolytic actions (Susarla et al., 2002). Runoff entering unvegetated conveyance structures resulting from ditch maintenance (e.g., dredging and mowing) may require contact with additional vegetated buffer strips for effective pesticide mitigation. Stoeckel et al. (1997) described microenvironments in these border strips that were found to slow migration and enhance degradation of pesticides from non–point source runoff. In addition to vegetated border strips, achievable BMPs may include a rotational maintenance regime in hydrologically connected drainage ditches to ensure efficacious water/macrophyte contact during agricultural runoff.

Although Runes et al. (2001) reported insignificant microbial degradation in wetland mesocosms, Walton and Anderson (1990) and Mirgain et al. (1993) reported that plant rhizospheres offered microbial activity that resulted in increased atrazine degradation through cometabolism. In this study, increased rhizosphere-associated microbial activity may have contributed to the absence of measurable sediment atrazine concentrations in the atrazine/*J. effusus* microcosm at 28 days. Further evidence of plant contribution to the uptake of atrazine by associated sediment was the 24-h sediment atrazine concentration in the pesticide combination/*J. effusus* microcosm, which was 43% and 65% lower than that in the combination/*L. peploides* and combination/unvegetated microcosms, respectively.

Plant pesticide concentrations in earlier microcosm studies utilizing the same pesticide exposure regime but containing mixed plant communities generally were found to be highest after 7 days (Bouldin, 2004); however, peak concentrations in the present study were measured at 24 h and dissipation of atrazine from the water column by 3 h. Although more rapid macrophyte sorption was measured in the present study, toxicity to aquatic test organisms remained in a vegetated microcosm through 14 days. This discrepancy between the two studies in the acute sensitivity measured may be explained by what Cairns and van der Schalie (1980) attributed to differences of scale. Microcosms incorporating vegetative communities were five times larger than those used in the present study. Increased water solubility may have contributed to higher plant atrazine uptake, and although evapotranspiration rates were not measured, the observed higher rates in microcosms containing J. effusus resulted in higher atrazine concentrations. However, more efficient mitigation by L. peploides was measured with higher plant pesticide concentrations and increased survival of aqueous test organisms at 24 h.

The benefit of having established plant communities within structures receiving agricultural runoff was illustrated by the persistence of aqueous toxicity in unvegetated microcosms. Milam et al. (2004) found that longer residence time in unvegetated wetlands reduced methyl parathion toxicity to *C. dubia, Hyalella azteca,* and *P. promelas.* Effective mitigation of exposure to methyl parathion in that study required 7 days for vegetated systems, whereas

14–28 days were necessary in unvegetated systems for a comparable reduction of toxicity. Therefore, parameters involving water residence time and complexity of vegetative communities become critical to analyzing the performance functions and treatment efficiency of conveyance structures, as with any wetland.

Pesticide combinations tended to exert greater toxicity in aquatic test organisms, suggesting an additive or synergistic effect with these mixtures. Similar findings of increased toxicity were observed with across-class mixtures of atrazine and organophosphates (Belden and Lydy, 2000; Anderson and Lydy, 2002). In addition to the consequence of greater toxicity in aquatic test organisms, using these combinations also resulted in higher measurable pesticide concentrations in the microcosms. Observed toxicity in microcosms of vegetated communities in an earlier study followed a pattern similar to that seen in this study (Bouldin, 2004). These concentrations may indicate that a pesticide has greater availability when it is part of a chemical mixture. The findings in this study showed (1) the importance of plants in the remediation of pesticides, (2) the resilience to and sorption of atrazine by J. effusus, and (3) the efficiency of L. peploides in mitigating the effects of pesticide exposure.

CONCLUSIONS

This research demonstrated the significance of vegetative attributes associated with agricultural receiving structures that aid in the mitigation of pesticide exposure. As an essential characteristic of these receiving areas, macrophytes offer not only increased binding sites for water-soluble chemicals in the water/plant interphase, but also sediment detritus, providing additional binding for less soluble compounds. Additional traits of aquatic vegetative communities include having associated rhizospheres supporting additional areas of pesticide exposure mitigation (Walton and Anderson, 1990; Mirgain et al., 1993). Although pesticide uptake has been measured in individual macrophyte species, differing chemical properties of pesticides and the co-occurrence of these chemicals in the environment may require the presence of diverse macrophyte communities or specific combinations to aid in the effective mitigation of pesticide exposure.

In addition to providing further information on the capability of vegetative communities associated with agricultural drainage structures to mitigate exposure, future studies could perhaps consider how to make attainable BMPs for the farm manager a reality. Therefore, research is needed to determine attainable edge-of-field designs, which would include in-place structures for the effective mitigation of agricultural runoff and practical maintenance of these BMPs.

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